**UW Medicine - Pathology**

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Movat's Stain (Modified) Procedure

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| Adopted Date: 09/10/97Review Date: 12/14/10Revision Date: 04/11/13 |

PURPOSE

To demonstrate collagen, reticular fibers, fibrin, elastic fibers, ground substance and mucin in connective tissue.

PROCEDURE

**Principle:**

Alcian blue is used to color acid mucopolysaccharide component of ground substance. The alkaline alcohol solution functions to convert the Alcian blue staining into issoluble monastral fast blue. Weigert's solution demonstrates the elastic tissue. Crocein scarlet-acid fuchsin stains muscle, cell cytoplasm, collagen and reticular fibers. The alcoholic saffron is used to demonstrate collagen and reticular fibers.

**Fixative:**

Acetic formalin sublimated recommended; neutral buffered formalin or Bouin's fluid may also be used.

**Microtomy:**

Cut paraffin sections at 4 um.

**Controls:**

Lung

**Reagents:**

**Alcian Blue, 1%**

Alcian blue 1.0 gm

Distilled water 100.0 gm

Solution stable for 6 months.

**Brown's Fluid**

Richard Allan Scientific. Ready made.

**Alkaline Alcohol**

95% ethanol 450 ml

Ammonium hydroxide, concentrated 50 ml

Solution stable for 3 months.

**Stock Weigert's Stain**

*Stock Solution:*

**Alcoholic hematoxylin, 2%**

Hematoxylin 10.0 gm

95% ethanol 500.0 ml

Solution stable for 3 months.

**Ferric Chloride**

Ferric chloride hexahydrate 12.4 gm

Distilled water 500.0 ml

HCl, conc. 5.0 ml

Solution stable for 6 months

**Iodine Solution**

Iodine 10.0 gm

Potassium iodide 20.0 gm

Distilled water 500.0 ml

Disolve the potassium in 10.0 ml of distilled water. Add the iodine and completely dissolve, then add the rest of the distilled water.

Solution stable for 6 months

*Working Solution:*

Hematoxylin, 2% 15 ml

Ferric chloride 10 ml

Iodine solution 5 ml

Mix in order given and use immediately. Discard solution after use.

**Crocein Scarlet - Acid Fuchsin**

*Stock Solution:*

**Crocein Scarlet**

Crocein scarlet M00 3B 1.0 gm

Distilled Water 99.5 ml

Acetic Acid concentrated 0.5 ml

Solution stable for 6 months

**Acid Fuchsin**

Acid fuchsin 0.1 gm

Distilled Water 99.5 ml

Acetic Acid concentrated glacial 0.5 ml

Solution stable for 6 months

*Working Solution:*

Crocein scarlet, stock solution 40.0 ml

Acid fuchsin, stock solution 10.0 ml

Solution stable for 3 months

**Glacial Acetic Acid, 1%**

Acetic acid, conc. glacial 1.0ml

Distilled water 99.0 ml

Solution stable for 3 months

**Phosphotungstic acid, 5%**

Phosphotungstic acid 5.0 gm

Distilled water 100.0 ml

**Saffron, alcoholic**

Saffron 6.0 gm

Absolute Ethanol 100.0 ml

Place in an air tight bottle in a 60oC oven for 48 hours to extract the saffron. Solution stable for 1 year.

**Staining Procedure:**

Use control slide.

1. Preheat Bouin’s solution in 60oC oven.
2. Deparaffinize and hydrate to distilled water.
3. Mordant in preheated Bouin's fluid for 30 minutes.
4. Remove and cool in slide rack for 5 minutes
5. Preheat Alkaline alcohol in 60oC oven.
6. Rinse in running water for 10 minutes.
7. 1% Alcian blue - 20 minutes.
8. Running water – 1 minute.
9. Preheated Alkaline alcohol - 10 minutes.
10. Running water - 2 to 5 minutes.
11. Weigert's - 60 minutes (do not use microwave).
12. Rinse in running water for 1 minute, then in distilled water for 1 minute.
13. Crocein Scarlett / Acid Fuchsin - 1 minute.
14. Rinse distilled water.
15. 5% phosphotungstic acid - 5 minutes.
16. Transfer directly to 1% acetic acid - 5 minutes.
17. Rinse well with distilled water.
18. Dehydrate 1 change 95% ETOH, 2 changes absolute ETOH - 1 minute each.
19. Alcoholic saffron - 7 minutes. Do not dump.
20. Rinse in 2 changes absolute ETOH - 1 minute each.
21. Clear in xylene and mount in Permount.

**Results:**

Nuclei and Elastic Fibers Black

Ground substance and Mucin Blue

Muscle Red

Collagen and Reticular Fibers Yellow

Fibrinoid and fibrin Intense Red

REFERENCES

Histology Laboratory, Virginia Mason Medical Center, Seattle, WA.

Sheehan, Hrapchak: *Theory and Practice of Histotechnology*. St. Louis, C.V. Mosby Co. 1980, pp 198-199.

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Written By: Director Approval:

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Histology Supervisor